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=> s integration and retrovir?

L1 2038 INTEGRATION AND RETROVIR?

=> s l1 and att

L2 20 L1 AND ATT

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L4 ANSWER 1 OF 6 MEDLINE DUPLICATE 1
AN 93131970 MEDLINE
DN 93131970
TI Site-directed mutagenesis of HIV-1 integrase demonstrates differential effects on integrase functions in vitro.
AU Leavitt A D; Shiue L; Varmus H E
CS Department of Immunology and Microbiology, University of California, San Francisco 94143-0502.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jan 25) 268 (3) 2113-9. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199304
AB The **retroviral** integrase (IN) protein is essential for **integration** of **retroviral** DNA into the host cell genome. To identify functional domains within the protein and to assess the importance of conserved residues, we performed site-directed mutagenesis of HIV-1 IN and analyzed the mutants in vitro for IN-mediated activities: 3' processing (**att** site-specific nuclease activity), strand transfer (the joining of **att** site oligonucleotides to target DNA), disintegration (the reverse of strand transfer), and **integration** site selection. Changing the conserved residue His-16 either to Cys or to Val in a proposed zinc-finger region had minimal effect on IN activities. Alteration of two highly conserved amino acid residues, Asp-116-->Ile and Glu-152-->Gly, each resulted in complete or nearly complete loss of 3' processing, strand transfer, and disintegration, whereas alteration of another conserved residue, Trp-235-->Glu, had no demonstrable effect on any of the activities in vitro. Two mutants, Asp-64-->Val and Arg-199-->Cys delta, each demonstrated differential effects on IN activities. Asp-64-->Val has no demonstrable strand transfer or disintegration activity yet maintains 3' processing activity at a diminished level. Arg-199-->Cys delta, which lacks part of the carboxyl terminus of IN, has impaired strand transfer activity without loss of disintegration activity. Use of a target site selection assay showed that all of our mutants with strand transfer activity maintain the same **integration** pattern as wild type IN. We conclude that not all highly conserved IN residues are essential for IN activities in vitro, zinc coordination by the proposed zinc-finger domain may not be required for the activities assayed, alteration of single residues can yield differential effects on IN activities, and target site selection into naked DNA is not necessarily altered by changes in strand transfer activity.

L4 ANSWER 2 OF 6 MEDLINE DUPLICATE 2
AN 92194474 MEDLINE
DN 92194474
TI Both substrate and target oligonucleotide sequences affect in vitro

integration mediated by human immunodeficiency virus type 1
 integrase protein produced in *Saccharomyces cerevisiae*.
 AU Leavitt A D; Rose R B; Varmus H E
 CS Department of Microbiology and Immunology, University of California,
 San Francisco 94143.
 SO JOURNAL OF VIROLOGY, (1992 Apr) 66 (4) 2359-68.
 Journal code: KCV. ISSN: 0022-538X.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 199206
 AB **Integration of retroviral DNA** into the host cell
 genome requires the interaction of **retroviral** integrase
 (IN) protein with the outer ends of both viral long terminal repeats
 (LTRs) to remove two nucleotides from the 3' ends (3' processing)
 and to join the 3' ends to newly created 5' ends in target DNA
 (strand transfer). We have purified the IN protein of human
 immunodeficiency virus type 1 (HIV-1) after production in
Saccharomyces cerevisiae and found it to have many of the properties
 described for **retroviral** IN proteins. The protein performs
 both 3' processing and strand transfer reactions by using HIV-1 or
 HIV-2 attachment (**att**) site oligonucleotides. A highly
 conserved CA dinucleotide adjacent to the 3' processing site of
 HIV-1 is important for both the 3' processing and strand transfer
 reactions; however, it is not sufficient for full IN activity, since
 alteration of nucleotide sequences internal to the HIV-1 U5 CA also
 impairs IN function, and Moloney murine leukemia virus **att**
 site oligonucleotides are poor substrates for HIV-1 IN. When HIV-1
att sequences are positioned internally in an LTR-LTR circle
 junction substrate, HIV-1 IN fails to cleave the substrate
 preferentially at positions coinciding with correct 3' processing,
 implying a requirement for positioning **att** sites near DNA
 ends. The 2 bp normally located beyond the 3' CA in linear DNA are
 not essential for in vitro **integration**, since mutant
 oligonucleotides with single-stranded 3' or 5' extensions or with no
 residues beyond the CA dinucleotide are efficiently used. Selection
 of target sites is nonrandom when **att** site
 oligonucleotides are joined to each other in vitro. We modified an
 in vitro assay to distinguish oligonucleotides serving as the
 substrate for 3' processing and as the target for strand transfer.
 The modified assay demonstrates that nonrandom usage of target sites
 is dependent on the target oligonucleotide sequence and independent
 of the oligonucleotide used as the substrate for 3' processing.

L4 ANSWER 3 OF 6 MEDLINE DUPLICATE 3
 AN 91111971 MEDLINE
 DN 91111971
 TI Moloney murine leukemia virus IN protein from disrupted virions
 binds and specifically cleaves its target sequence in vitro.
 AU Ishimoto L K; Halperin M; Champoux J J
 CS University of Washington, School of Medicine, Department of
 Microbiology, Seattle 98195.
 NC CA51605 (NCI)
 5T32CA09229-12 (NCI)
 SO VIROLOGY, (1991 Feb) 180 (2) 527-34.
 Journal code: XEA. ISSN: 0042-6822.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English

FS Priority Journals; Cancer Journals
 EM 199105
 AB The **integration** of **retroviral** DNA plays an essential role in the viral life cycle. Previous studies of the Moloney murine leukemia virus (M-MuLV) have shown that viral **integration** is mediated by the integrase (IN) protein acting on the 13-bp inverted repeats that flank the linear viral DNA produced during reverse transcription. Prior studies have also shown that when the M-MuLV IN protein is produced in *Escherichia coli* it retains an ability to specifically associate with the viral inverted repeats (Krogstad and Champoux, 1990). In this study we present evidence that the IN protein present in detergent-disrupted virions is capable of specifically interacting with double-stranded oligonucleotides that correspond to the viral inverted repeats, and that this interaction may change after **integration**-related processing of the viral **att** sites. We further present evidence that, in vitro, detergent-disrupted virions are capable of specifically cleaving ds-IR oligonucleotides in an IN-dependent reaction that mimics the trimming step that precedes **integration**.

L4 ANSWER 4 OF 6 MEDLINE DUPLICATE 4

AN 89259068 MEDLINE

DN 89259068

TI **Retrovirus** vectors containing an internal attachment site: evidence that circles are not intermediates to murine **retrovirus integration**.

AU Ellis J; Bernstein A

CS Department of Medical Genetics, University of Toronto, Ontario, Canada..

SO JOURNAL OF VIROLOGY, (1989 Jun) 63 (6) 2844-6.

Journal code: KCV. ISSN: 0022-538X.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 198909

AB Murine cells were infected with a **retrovirus** vector containing a defective native attachment (**att**) site, an internal **att** site, and a neo gene. Analysis of the proviruses by virus rescue and Southern blots demonstrated that internal **att** sites were not utilized for **integration** and could not complement defects in the native site. These data suggest that murine **retroviruses** do not integrate in vivo through tandem long terminal repeat circular DNA intermediates.

L4 ANSWER 5 OF 6 MEDLINE DUPLICATE 5

AN 87151100 MEDLINE

DN 87151100

TI The spleen necrosis virus int gene product expressed in *Escherichia coli* has DNA binding activity and mediates **att** and U5-specific DNA multimer formation in vitro.

AU Luk K C; Gilmore T D; Panganiban A T

NC P30-CA07175 (NCI)

P01-CA22443 (NCI)

SO VIROLOGY, (1987 Mar) 157 (1) 127-36.

Journal code: XEA. ISSN: 0042-6822.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English
 FS Priority Journals; Cancer Journals
 OS GENBANK-M16609
 EM 198706
 AB To facilitate the in vitro study of the spleen necrosis virus (SNV) int gene product, we expressed the viral int locus in an Escherichia coli expression vector. Antiserum made against the protein produced in bacteria precipitated a 44-kDa polypeptide from virus-infected chicken embryo fibroblasts. This result is consistent with the expected size of the SNV int polypeptide. In a protein blotting assay, the expressed protein binds strongly to DNA and was able to complex nonspecifically with both single- and double-stranded DNAs containing or lacking viral sequences. However, under solution conditions favoring transient DNA unwinding, DNA binding was confined to supercoiled molecules containing either the SNV **att** sequence (the viral cis-acting region required for **integration**) or the U5 region of the long terminal repeat alone. Under these conditions of specific binding, multimeric DNA species were formed by apparent intermolecular interaction between protein-DNA complexes. These data indicate that **retroviral integration** may require local DNA unwinding at the **att** site for complex formation between the int gene product and DNA. This complex may be an intermediate in the viral DNA insertion process.

L4 ANSWER 6 OF 6 MEDLINE
 AN 85088512 MEDLINE
 DN 85088512
 TI The **retrovirus** pol gene encodes a product required for DNA **integration**: identification of a **retrovirus** int locus.
 AU Panganiban A T; Temin H M
 SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1984 Dec) 81 (24) 7885-9.
 Journal code: PV3. ISSN: 0027-8424.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 198504
 AB We mutagenized cloned spleen necrosis virus DNA to identify a region of the **retrovirus** genome encoding a polypeptide required for **integration** of viral DNA. Five plasmids bearing different lesions in the 3' end of the pol gene were examined for the ability to integrate or replicate following transfection of chicken embryo fibroblasts. Transfection with one of these DNAs resulted in the generation of mutant virus incapable of integrating but able to replicate at low levels; this phenotype is identical to that of mutants bearing alterations in the cis-acting region, **att**. To determine whether the 3' end of the pol gene encodes a protein that interacts with **att**, we did a complementation experiment. Cells were first infected with an **att**- virus and then superinfected with the **integration**-deficient virus containing a lesion in the pol gene and a wild-type **att** site. The results showed that the **att**- virus provided a transacting function allowing **integration** of viral DNA derived from the mutant bearing a wild-type **att** site. Thus, the 3' end of the pol gene serves as an "int" locus and encodes a protein mediating **integration** of **retrovirus** DNA through interaction

with att.

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